

Inhibiting the effect of ^{90}Sr - ^{90}Y ophthalmic applicators on rat corneal neovascularization induced by sutures

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Abstract

• **AIM:** To investigate a practical technique used to inhibit corneal angiogenesis with a ^{90}Sr - ^{90}Y ophthalmic applicator.

• **METHODS:** A ^{90}Sr - ^{90}Y ophthalmic applicator was detected with a radioactive nuclide application treatment healthy protection standard. The applicator used was produced through medical dosimetry research; it had a concave applicator and measured the applicator temperature, serviceable humidity range, applicator appearance status, applicator radiation homogeneity, radioautography, and radiological safety of the original applicator surface. A vessel model was established using newborn rats, with sutures around the corneal limbus. Corneal neovascularization (CNV) were observed with a slit lamp. The new vessel length and response area were measured.

• **RESULTS:** Low-dose radiation can inhibit CNV after corneal sutures. The absorbed dose of the applicator (0.046 Gy/s) was safe for the treatment of it. The lengths of new vessels and the areas of new vessels were lower than the new born vessel rat group ($P < 0.01$).

• **CONCLUSION:** The optimal radiation dose emitting from the applicator can be safe and potentially used in humans.

• **KEYWORDS:** radiation; cornea; neovascularization

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INTRODUCTION

Corneal neovascularization (CNV) leads to decreased corneal transparency, which can induce vision

impairment and even lead to blindness. The complex process of CNV is related to multiple factors. Angiogenesis is a highly organized sequence of cellular events stemming from vascular initiation, formation, maturation, remodelling and regression processes controlling and modulating tissue requirements [1]. Pathological CNV caused by infectious, traumatic and degenerative diseases depends on the imbalance of angiogenic and anti-angiogenic factors [2]. Although many studies have described how to treat newborn corneal vessels, no exact method has been detailed until now. New therapeutic strategies must be explored. $^{90}\text{Sr}/^{90}\text{Y}$ is a key example of a high-beta-energy-emitting radionuclide that is available from the strontium-90 (^{90}Sr)/ ^{90}Y radionuclide generator system [3-5]. Using such electrochemical techniques, the lower consumption of reagents and minimal generation of radioactive waste are compatible with "green chemistry" principles [6]. Radium beta emitters involving $^{90}\text{Sr}/^{90}\text{Y}$ applicators have been widely used to treat superficial skin haemangioma in early childhood [7].

This study aimed to investigate the effects of brachytherapy with low-dose beta radiation emitted by $^{90}\text{Sr}/^{90}\text{Y}$ on CNV in a model using sutures and newborn rat vessels.

MATERIALS AND METHODS

^{90}Sr - ^{90}Y Ophthalmic Applicator Design The applicator used in this experiment is routinely used in clinical nuclear medicine. It was purchased from the Atomic Energy Isotope Research Institute (China), and it was detected using radioactive nuclide application treatment healthy protection standards. The applicator that was used was produced through medical dosimetry research; it had a concave applicator and measured the applicator temperature, serviceable humidity range, applicator appearance status, applicator radiation homogeneity, radioautography, and radiological safety of the original applicator surface.

Newborn Rat Vessel Model with Sutures This study followed the guidelines of the Guide for the Care and Use of Laboratory Animals, as well as the principles of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Two-month-old female Wistar rats without eye diseases (Animal Experiment Department, Jilin University, China) weighing 200-250 g were anesthetized with a 1% pentobarbital sodium intraperitoneal injection (1.3 mg/kg). We established a newborn rat vessel model with sutures (34 rats). Three 10/0 nylon sutures were applied to the limbus of the cornea.

Observation and Examination The 54 newborn rats were randomly divided into three groups: the control group (radiation treatment without sutures, $n=17$), the suture group (no radiation treatment, $n=17$) and the irradiation treatment with sutures group (IT, $n=17$). Rats in the IT and control groups received a low dose of 7 Gy once daily for 7 consecutive days. All rats in each group were clinically evaluated. At the end of the experiment, all rats were sacrificed with an overdose of 1% pentobarbital sodium. The corneas of the rats were harvested, and only the right eye of each rat was used. We randomly selected six rats from each group, and we observed the neovascularization using a slit lamp. We calculated the new vessel length and response area using the Needleman *et al* [8] method. The data were statistically analysed.

Measurement of the Applicator A SSR9013 applicator (Figure 1) was used; the absorbed dose rate was 0.046 Gy/s, and the absorbing dose rate was 0.0345 Gy/s. The applicator measurement followed the radioactive nuclide application treatment for health protection standard.

Manufacture research: the spherical source was made by nuclide ^{90}Sr - ^{90}Y . The applicator was fit to the corneal spherical surface. Medical dosimetry research: the dosimetric measurement and spatial distribution of the applicator were useful for determining clinical needs. The applicator concave surface contamination level was less than 185 Bq. The applicator temperature was 5°C - 40°C , and the serviceable humidity range was less than 80%. In terms of the applicator appearance status, the applicator had an integrated surface, and no radioactive substance was divulged. Radioautography: the radioactive source surface of the ophthalmic applicator was placed above the sensitization film and then removed after 1 to 5s. The analysis of the printed film showed the homogeneity of the applicator (Figure 2).

Statistical Analysis The lengths and areas of the new vessels were analysed, followed by one-factor analysis of variance to compare the between-group differences, and $P < 0.05$ was considered to be statistically significant.

RESULTS

Measurement of New Vessel Length for Corneal Neovascularization Using the Needleman *et al* [8] method, six rats were randomly selected from each group on the 7th day. The average new vessel length (VL) was calculated as follows. The cornea was divided into four quadrants. The values of the longest vessel from the four quadrants were summed, and the average equalled the VL value. The IT group manifested a VL value that was significantly lower than that of the newborn rat vessel model group. The statistical analysis was followed by one-factor analysis of variance, and $P < 0.01$ was considered to be statistically significant compared to the newborn rat vessel model group (Figure 3).

Measurement of New Vessel Area for Corneal Neovascularization Following the Needleman *et al* [8] method,

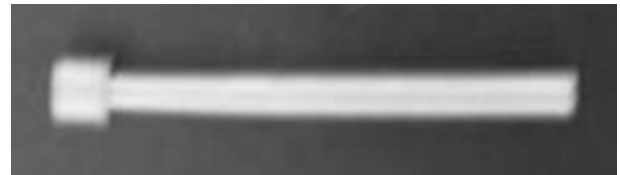


Figure 1 An ^{90}Sr - ^{90}Y ophthalmic applicator This SSR9013 applicator was consistent with the radioactive nuclide application treatment health protection standard.

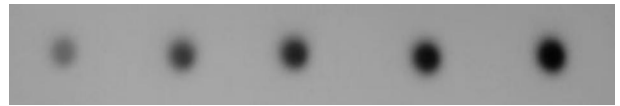


Figure 2 Radioautography The radioactive source surface of the ophthalmic applicator was placed above the sensitization film and then removed after 1 to 5s. The ophthalmic applicator's autoradiography results showed homogeneity. The radiant security of the applicator: the maximum beta radiation level emitted by the $^{90}\text{Sr}/^{90}\text{Y}$ applicator was 0.546 MeV. The range of 0.546 MeV in the tissue was 0.246 cm. The distance between the applicator and the cornea was 1 mm, and the central corneal thickness was 0.6 mm. The average anterior chamber depth was 0.27 mm. Therefore, the dosage used in this experiment was safe enough to eliminate cataracts and relative complications.

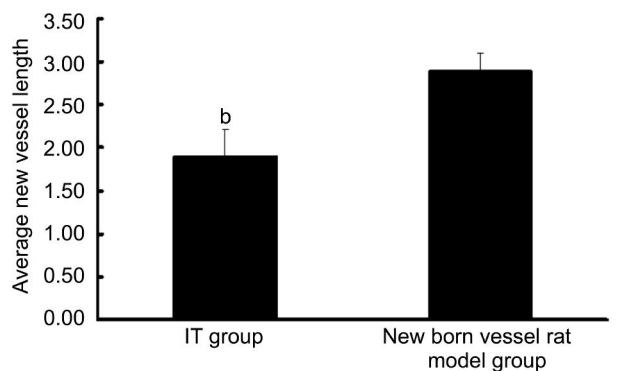


Figure 3 The IT group manifested a VL value that was significantly lower than that of the newborn rat vessel model group $^bP < 0.01$ was considered to be statistically significant compared to the newborn rat vessel model group.

six rats were randomly selected from each group on the 7th day. The average new vessel area was calculated using the following formula: $\text{area (mm}^2\text{)} = \text{CH}/12 \times 3.14 [r^2 - (r - \text{VL})]$; $r = 3$ mm; CH measured the hours of new vessels. The value of the new vessel area in the IT group was much lower than that in the newborn rat vessel model group. The statistical analysis was followed by one-factor analysis of variance, and $P < 0.01$ was considered to be statistically significant compared to the newborn rat vessel model group (Figure 4). The CNV length and area were much lower in the IT group compared with the newborn rat vessel model on the seventh day after the experiment (length: $P < 0.01$; area: $P < 0.01$). The statistical analysis was followed by one-factor analysis of variance, and $P < 0.01$ was considered to be statistically significant compared to the newborn rat vessel model group (Figures 3, 4).

Western Blot Analysis Proteins were extracted from the rat corneas on the 7th day after suturing. Equal amounts of proteins extracted from lysates were subjected to electrophoresis on 10% Tricine gels and then electrophoretically transferred to PVDF membranes. After 1h of blocking in 0.05 g/mL milk, the blots were incubated with primary antibodies against vascular endothelial growth factor (VEGF) at 4°C overnight. After washing three times with Tris-buffered saline with 0.05% Tween 20 for 10min each, the membranes were incubated with horseradish Peroxidase (HPR)-conjugated goat anti-rabbit IgG for 1h at room temperature. The specific bands were visualized using enhanced chemiluminescence reagents and recorded on film (Figure 5).

DISCUSSION

Angiogenesis, the process by which new blood vessels arise from pre-existing vessels, is a critical part of many disease processes, including CNV [9-12]. Regulation of angiogenesis is crucial for many diseases. Antiangiogenic therapy focusing on the tumour microenvironment is a traditional approach for treating cancer [13]. Angiogenesis is a complex process that includes multiple cell types, cytokines, adhesion molecules, growth factors, and signal transduction during inflammation [14]. Radiotherapy for surgery and tumour therapy was developed more than a century ago. Higher doses of radiotherapy that minimize absorbance by normal tissues will be the next development trend [15]. Radiation damage to ocular tissues includes conjunctivitis, eyelid lesions, keratitis, and keratoconjunctivitis sicca [16]. Appropriate radiation therapy has long been attempted for ocular diseases. Intraocular tumours located in the iris, ciliary body, and choroid could be treated with plaque brachytherapy, except for tumours with orbital extension and no light perception vision [17].

Endothelial dysfunction has been associated with a number of pathophysiological processes. VEGF, which is synthesized and released by endothelial cells, regulates angiogenesis, vascular tone and permeability [18-20]. The formation of CNV is dependent upon VEGF, as well as the proliferation of vascular endothelium, remodelling of extracellular matrix components and the activation of cytokines [21]. VEGF plays a major role in the process of vessel branch formation, leading to aberrant angiogenic responses. It also plays important roles in many diseases [22-24]. Radium applicators and pure beta emitters have been widely used in the past to treat skin haemangioma in early childhood [25]. High expression levels of VEGF have been associated with a poor prognosis in cancer patients, indicating that VEGF could be linked to the efficacy of radiotherapy [26]. VEGF is a valuable molecular marker in treatment outcomes following radiation therapy for rectal adenocarcinoma [27]. Radiation therapy can also provide a dose-dependent benefit in the treatment of neovascular age-related macular degeneration, which can reduce the frequency of anti-VEGF injections to maintain visual acuity [28]. The side effects of radiotherapy prevent widespread

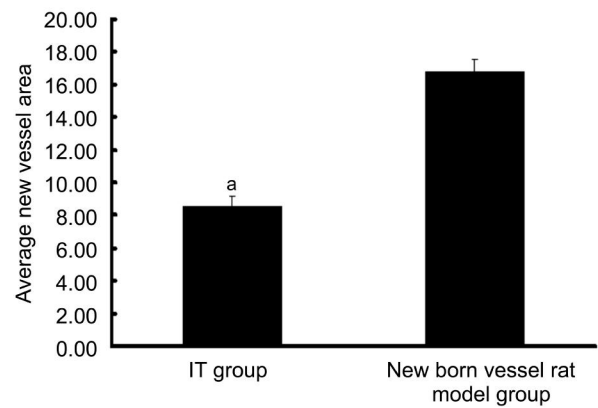


Figure 4 The IT group manifested an average new vessel area that was significantly lower than that of the newborn rat vessel model group ^a $P < 0.05$ was considered to be statistically significant compared to the newborn rat vessel model group.

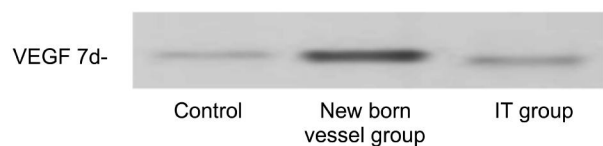


Figure 5 Effect of irradiation on VEGF expression after corneal suturing The Western blot analysis results showed that VEGF was expressed in normal rat corneas and that this expression increased from day 3 and peaked at day 7. According to the data, the VEGF expression was much lower in the irradiation group compared to the alkali burn group on the 7th day.

radiotherapy usage in ocular disease. In this study, we investigated the potential role of low-dose radiation for CNV therapy. CNV is a major cause of blindness and can lead to keratoplasty failure. We focused on the available therapeutic options for CNV. The effect of ⁹⁰Sr-⁹⁰Y ophthalmic applicator on CNV was unknown. Based on the data from our investigation, low doses of beta emissions divided several times showed good outcomes. The inhibition effect peaked on the 7th day after irradiation therapy. The irradiation distance is safe; it cannot induce cataract and corneal opacity. There were significant differences in the average new vessel length and new vessel area in the suturing group and the IT group, as we predicted. We made these observations using lamp light, and we found that divided low-dose radiation performed better than high-dose radiation or therapy only once (data not shown). The corneas were transparent, without vessels. The vessels terminated at the corneal limbus. Angiogenesis occurs in corneal pathologies and wounds, and new vessels leak easily. Exudation and fibrosis of new vessels can lead to blindness. It is hard to treat CNV. Until now, there has been no definitive approach. Radiation can inhibit tumour neovascularization, particularly in neonatal angioma. A suitable dose of radiation can be safe enough to inhibit the CNV induced by sutures. The therapeutic effect of CNV is unclear.

CNV occurs in corneal injury pathologies, such as infection, chemical burns, physical trauma, and corneal transplant

rejection [29-30]. The data above clearly suggest that radiation therapy can be used to treat CNV. ⁹⁰Sr-⁹⁰Y ophthalmic applicators may provide new insight into the treatment of angiogenic ocular surface diseases. Therefore, further studies are needed to determine the appropriate dosage and the precise mechanism of the inhibiting effect of irradiation. In conclusion, this study demonstrates that radiation may be a new pragmatic method to treat corneal vessels. Thus, we conclude that ⁹⁰Sr-⁹⁰Y ophthalmic applicators have the potential to become an ideal platform for CNV therapy.

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